PB86-212628

Cytogenetic Characterization of the L5178Y TK+/-3.7.2C Mouse Lymphoma Cell Line

(U.S.) Health Effects Research Lab.
Research Triangle Park, NC

1985

U.S. Department of Commerce
National Technical Information Service
NTIS

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before comr

1. REPORT NO.	2.	PB86-212628
EPA/600/J-85/403		

4. TITLE AND SUBTITLE
Cytogenetic characterization of the L5178Y TK$^{+/-}$ 3.7.2C mouse lymphoma cell line
(Journal Version)

5. REPORT DATE
1985

6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)
Jeffrey Sawyer[1], Martha M. Moore[2], Donald Clive[3] and John Hozier[1]

8. PERFORMING ORGANIZATION REPORT NO.

9. PERFORMING ORGANIZATION NAME AND ADDRESS
[1]Med. Genetics Lab., Depart. of Biological Sciences FL Inst. of Tech., Melbourne, FL 32901; [2]Genetic Toxicology Div. HERL, USEPA, RTP, NC 27711; [3]Genetic Tox. Lab., Burroughs Wellcome Co., RTP, NC 27709

10. PROGRAM ELEMENT NO.

11. CONTRACT/GRANT NO.

12. SPONSORING AGENCY NAME AND ADDRESS
Health Effects Research Laboratory (HERL)
Office of Research and Development (ORD)
U.S. Environmental Protection Agency (USEPA)
Research Triangle Park, North Carolina 27711

13. TYPE OF REPORT AND PERIOD COVERED
Journal Article

14. SPONSORING AGENCY CODE
EPA 600/11

15. SUPPLEMENTARY NOTES
Mutation Research 174, Issue #5, Oct. 1985, 243-253

16. ABSTRACT

The cytogenetic characterization of the L5178Y TK$^{+/-}$ 3.7.2C mouse lymphoma cell line was carried out, utilizing G-banded metaphase chromosomes, to provide a karyotypic basis for the precise delineation of induced rearrangements in TK$^{+/-}$ mutants. Band-pattern measurements were used to construct ideograms which represent the position, number, size and staining intensity of the chromosome bands. The TK$^{+/-}$ 3.7.2C cell line has been shown to provide quantitation of forward mutations induced at the autosomal thymidine kinase (TK) locus in this cell line. Chromosome analysis of the TK$^{+/-}$ 3.7.2C cell line and derived TK$^{-/-}$ mutants has become important in demonstrating that the TK$^{+/-} \rightarrow$ TK$^{-/-}$ assay may detect and distinguish between chromosomal events and smaller, perhaps point-mutation, events in mutant colonies.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES
RELEASE TO PUBLIC	UNCLASSIFIED	12
	20. SECURITY CLASS (This page) UNCLASSIFIED	22. PRICE

EPA Form 2220-1 (Rev. 4-77)

REPRODUCED BY
NATIONAL TECHNICAL INFORMATION SERVICE
U.S DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

EPA/600/J-85/408
JOURNAL ARTICLE

Mutation Research, 147 (1985) 243–253
Elsevier

MTR 08589

Cytogenetic characterization of the L5178Y TK$^{+/-}$ 3.7.2C mouse lymphoma cell line

Jeffrey Sawyer [1], Martha M. Moore [2], Donald Clive [3] and John Hozier [1]

[1] *Medical Genetics Laboratory, Department of Biological Sciences, Florida Institute of Technology, Melbourne, Fl 32901,* [2] *Mutagenesis and Cellular Toxicology Branch, Genetic Toxicology Division, Health Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711 and* [3] *Genetic Toxicology Laboratory, Burroughs Wellcome Company, 3030 Cornwallis Road, Research Triangle Park, NC 27709 (U.S.A.)*

(Received 25 June 1984)
(Revision received 6 March 1985)
(Accepted 9 April 1985)

Summary

The cytogenetic characterization of the L5178Y TK$^{+/-}$ 3.7.2C mouse lymphoma cell line was carried out, utilizing G-banded metaphase chromosomes, to provide a karyotypic basis for the precise delineation of induced rearrangements in TK$^{-/-}$ mutants. Band-pattern measurements were used to construct ideograms which represent the position, number, size and staining intensity of the chromosome bands. The TK$^{+/-}$ 3.7.2C cell line has been shown to provide quantitation of forward mutations induced at the autosomal thymidine kinase (TK) locus in this cell line. Chromosome analysis of the TK$^{+/-}$ 3.7.2C cell line and derived TK$^{-/-}$ mutants has become important in demonstrating that the TK$^{+/-}$ → TK$^{-/-}$ assay may detect and distinguish between chromosomal events and smaller, perhaps point-mutation, events in mutant colonies.

The L5178Y TK$^{+/-}$ mouse lymphoma mutagen assay allows quantitation of forward mutations at the autosomal thymidine kinase locus (Clive and Spector, 1975; Clive and Voytek, 1977; Clive et al., 1979). In a typical test procedure, TK$^{+/-}$ cells are treated with a range of doses of the test substance. The cells are incubated, washed free of tests substance and allowed time for the phenotypic expression of newly induced mutations (Moore and Clive, 1982). Treated cells are cloned in soft agar in trifluorothymidine selection medium that only allows growth of TK$^{-/-}$ mutant colonies (Moore-Brown et al., 1981).

The most striking morphological property of the mutant colonies following 9–11 days of incubation at 37°C is a bimodal frequency distribution of colony sizes for most mutagenic substances tested. This bimodal distribution is quite distinct in soft agar cloning medium, consisting of a small-colony (σ) and a large-colony (λ) mutant peak (Clive et al., 1979).

Recently, it has been shown that chromosomal aberrations occur in σ mutant colonies in the L5178Y TK$^{+/-}$ mutation assay system but not in λ mutants (Hozier et al., 1981, 1982). These aberrations seem to be implicated in the slow-growth kinetics associated with small-colony mutants (Moore and Clive, 1982) and consistently involve chromosome 11, the known location of the thymidine kinase gene in the mouse (Kozak and Ruddle, 1977).

Until now the precise extent of these induced aberrations could not be ascertained because of the lack of standardization of the chromosome

0165-1161/85/$03.30 © 1985 Elsevier Science Publishers B.V. (Biomedical Division)

NTIS is authorized to reproduce and sell this report. Permission for further reproduction must be obtained from the copyright owner.

constitution of the L5178Y $TK^{+/-}$ 3.7.2C cell line. The purpose of this study is to precisely characterize the chromosomal banding pattern of the $TK^{+/-}$ 3.7.2C cell line in order to delineate the nature of the rearrangements found in the mutant colonies. In addition, the availability of a standard karyotype for the L5178Y $TK^{+/-}$ 3.7.2C cell line should allow comparison of cytogenetic results among various laboratories using the $TK^{+/-} \rightarrow TK^{-/-}$ assay system.

Materials and methods

Cell culture and chromosome preparation

L5178Y/$TK^{+/-}$ 3.7.2C cells were maintained in suspension culture with Fischer's Medium for Leukemic Cells of Mice supplemented with 10% horse serum, Pluronic F68 and sodium pyruvate (Clive and Spector, 1975; amended by Clive et al., 1979). Maintenance cultures were counted daily and diluted to a density of 0.3×10^6 cells/ml, or more recently 0.2×10^6 cells/ml. On weekends, cultures were routinely diluted to approximately $5-7 \times 10^3$ cells/ml to keep cells growing in log phase (Moore and Clive, 1982). Once a week, selection against spontaneous $TK^{-/-}$ mutants was accomplished by adding THMG (thymidine + hypoxanthine + methotrexate + glycine) to cultures (Clive and Spector, 1975). Under these conditions, the karyotype of the cell line appears to be stable (Hozier et al., 1981).

The THC protocol (trypsin/hypotonic/colcemid) for human leukemia chromosomes (Hozier and Lindquist, 1980) was adapted for L5178Y cells. 10 or 15 ml of cell-culture suspension was placed in a 15-ml conical bottom plastic centrifuge tube. The cell suspension was cooled on ice for 5 min, followed by centrifugation at $200 \times g$ for 5 min at room temperature. The supernatant was poured off, leaving 0.2–0.3 ml over the cell pellet. The pellet was resuspended with gentle agitation. THC chromosome buffer (consisting of 1 ml trypsin–EDTA (Gibco No. 610-5300), 9 ml 75 mM KCl, and 80 µl of 10 µg/ml colcemid) was added in steps: 1 ml of THC was added with gentle agitation, followed by 9 ml with further agitation. The cell suspension in THC was incubated at 37°C for 20 min in a water bath. Cells were centrifuged as before, leaving 0.2–0.3 ml of THC over the pellet, followed by resuspension by gentle agitation. 1 ml of freshly made fixative (3:1, methanol:acetic acid) was added and mixed by agitation followed by 7 ml additional fixative. The suspension was incubated for 20 min at room temperature. Centrifugation and fixation steps were repeated at least 3 times.

Cell suspension was dropped onto slides and pretreated using the HCl–formamide procedure and stained with Wright's stain (Hozier et al., 1982). Briefly, chromosome preparations were banded by being placed in 1 N HCl for 20 min, followed by a 90-min incubation in 50% formamide and $2 \times$ SSC. The slides were then rinsed in tap water and dehydrated in 95% ethanol for 30 min. Following dehydration and a 2-h room-temperature drying period, the slides were stained with a 3:1 phosphate buffer/Wright's stain mix. G-banded chromosome preparations were photographed at a primary magnification of $1000 \times$, using Kodak technical pan film No. 2415. Chromosomes were cut out, aligned and mounted. 5 individual chromosomes of each type were then re-photographed and enlarged $5 \times$ for measurements.

Construction of the karyotype and control of bias

To control for possible bias in the choice of chromosomes, mitotic cells included in the analysis were chosen on the basis of quality of the overall banding patterns rather than for the appearance of any given individual chromosome. The chromosomes shown in Fig. 3 are representative for the entire cell population. For band measurements, the 5 samples of each chromosome were measured to the nearest millimeter from the enlarged photographs. The total length of each chromosome and the relative size of each visible band were measured. The band widths were converted into the percentage of total chromosome length by dividing the width of each band by the chromosome length (Mainguy et al., 1975). The sizes of bands for each distinguishable chromosome homologue were calculated separately.

Chromosome-band assignments were made in accordance with the Committee on Standardized Genetic Nomenclature for Mice (1972). The region including the centromere is labelled region 'A' followed by region 'B' and so forth, after the

demarcation of landmark bands. Bands within regions are numbered consecutively with the band closest to the centromere being given the lowest number.

Certain homologous pairs of $TK^{+/-}$ 3.7.2C chromosomes show heteromorphic centromeres or other chromosomal distinctions. Therefore, a suffix a or b is used to distinguish the homologous chromosomes.

Derivative chromosomes of unknown origin are designated as 'marker' chromosomes. These are chromosomes which are morphologically distinct from normal but appear consistent in their own banding patterns. Regions of these chromosomes are designated by dotted lines, as are translocations of unknown origin and tentatively assigned chromosomes (see Results).

Results

A composite karyotype of the L5178Y $TK^{+/-}$ 3.7.2C cell line is presented in Fig. 1. The G-banding patterns reveal a number of chromosomal rearrangements which are characterized in ideogram form in Fig. 2 and discussed in detail below with reference to sets of examples for each chromosome (Fig. 3).

Data obtained from chromosome measurements of the DBA/2 mouse, which was the original source of L5178Y mouse-lymphoma cells, indicate that the assignments of chromosomal lengths for homologous pairs versus chromosome number in this organism are inconsistent in some cases with the normal mouse standard karyotype (Sawyer and Hozier, unpublished). As with human chromosomes, mouse chromosomes have been assigned numbers based on overall length, with the largest chromosome having the lowest number (Nesbitt and Franke, 1973). We note that the mouse-chromosome nomenclature contains several incorrectly assigned chromosomes according to their actual lengths. In this regard, measurements indicate that with respect to the normal homologues in the L5178Y $TK^{+/-}$ cell line, chromosome 9 is longer than chromosome 8 (Fig. 1). Also chromosome 12 is larger than chromosomes number 7–11. The chromosome 15 is larger than 14 and chromosome 17 is larger than chromosome 16.

When considering the total diploid chromosome length, we find that the extra material represented by the marker chromosomes closely matches the amount lost by the deletion in chromosome 14b, and the absence of one of the homologues of the chromosome 15. Thus it appears that the total chromosomal material present in the $TK^{+/-}$ 3.7.2C cell line is nearly equal to that present in the normal mouse karyotype.

Comparison of the $TK^{+/-}$ 3.7.2C cell line karyotype with the normal mouse (DBA/2) indicates the presence of several major structural rearrangements. These changes will be discussed with the DBA/2 karyotype being considered as normal since, as previously mentioned, it was the origin of the mouse lymphoma cells used in this assay.

Chromosomes number 1 of the $TK^{+/-}$ 3.7.2C cell line both appear morphologically unchanged except for a centromeric heteromorphism in one of the two homologues. The chromosome with the heteromorphism is slightly longer than normal (Fig. 3a).

The chromosomes 2 (Fig. 3a) both appear unchanged from the DBA/2 karyotype as do the chromosomes 3. The chromosomes 4 of the $TK^{+/-}$ 3.7.2C line (Fig. 3a) have one normal homologue and one with a segment translocated onto the distal end. The translocated segment contains two G-positive bands and one G-negative band.

The $TK^{+/-}$ karyotype contains one normal chromosome 5 (Fig. 3a), and a chromosome 5 with bands A1–B1 translocated to the chromosome 15. There is one normal chromosome 6 in the $TK^{+/-}$ cell line (6a) and another chromosome tentatively identified as its partially deleted homologue (6b). Chromosome 6b is deleted at band 6D1 (Fig. 3a).

Chromosomes 7 consist of one normal homologue (7a) and one abnormal homologue (7b) with a centromeric heteromorphism and a paracentric inversion involving bands 7D2–7F2. The centromeric heteromorphism in the inverted 7 makes this chromosome slightly longer than the normal homologue (Figs. 3a and 3b).

Both chromosomes 8 appear normal in the $TK^{+/-}$ 3.7.2C cell line (Fig. 3b). The chromosomes 9 have one normal homologue (9a) and one abnormal homologue (9b) containing a transloca-

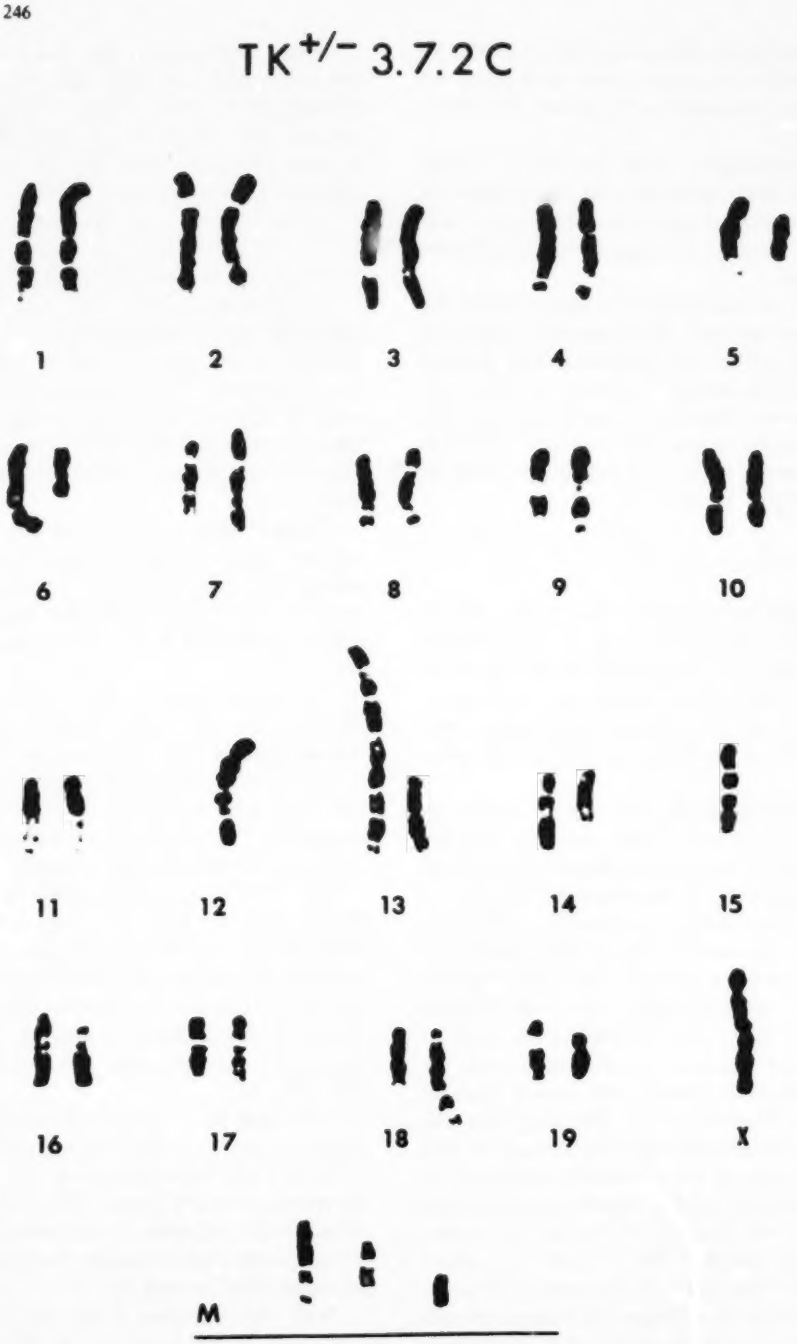

Fig. 1. The L5178Y TK$^{+/-}$ 3.7.2C cell line karyotype. Note presence of at least 1 homologue for each chromosome pair. Marker chromosomes (M) are of unknown origin.

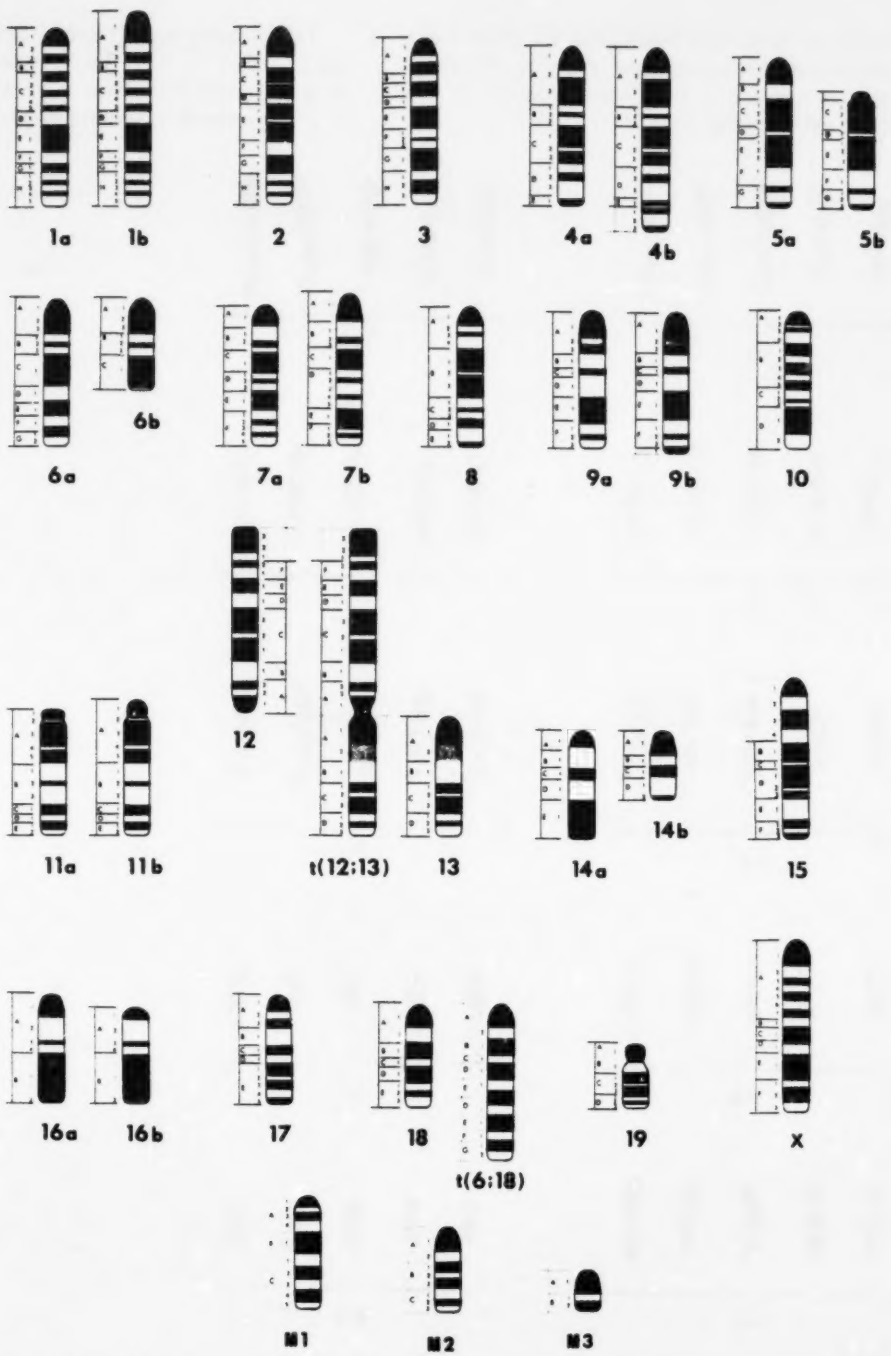

Fig. 2. Chromosome ideograms for the TK$^{+/-}$ 3.7.2C cell line.

tion of one G-positive band onto the distal band (band F3) of the chromosome (Fig. 3b). The chromosomes number 10 both appear normal in the $TK^{+/-}$ cell line (Fig. 3b).

The chromosomes 11 are of primary interest in the $L5178Y/TK^{+/-} \rightarrow TK^{-/-}$ mutagen assay. The only detectable difference in the chromosomes 11 is a centromeric heteromorphism in one of the

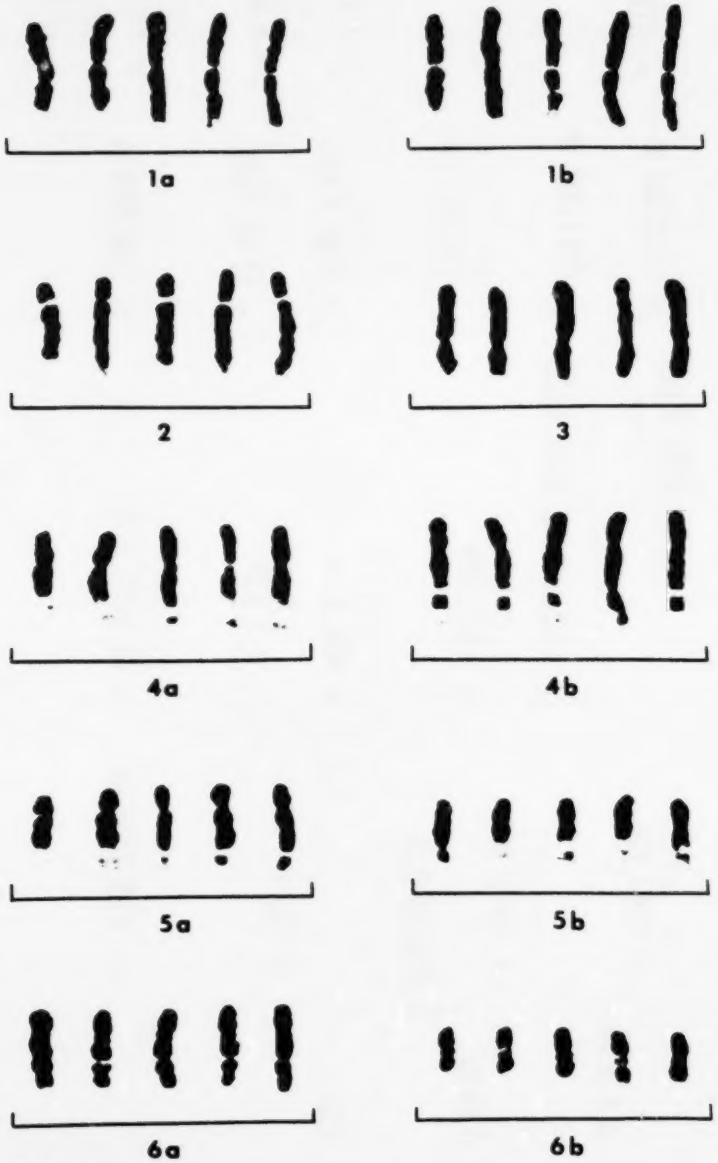

Fig. 3(a).

Fig. 3. Representative chromosomes of each distinguishable type. (a) Chromosomes 1a–6b. (b) Chromosomes 7a–13. (c) [illegible] 14a–X and M1–3.

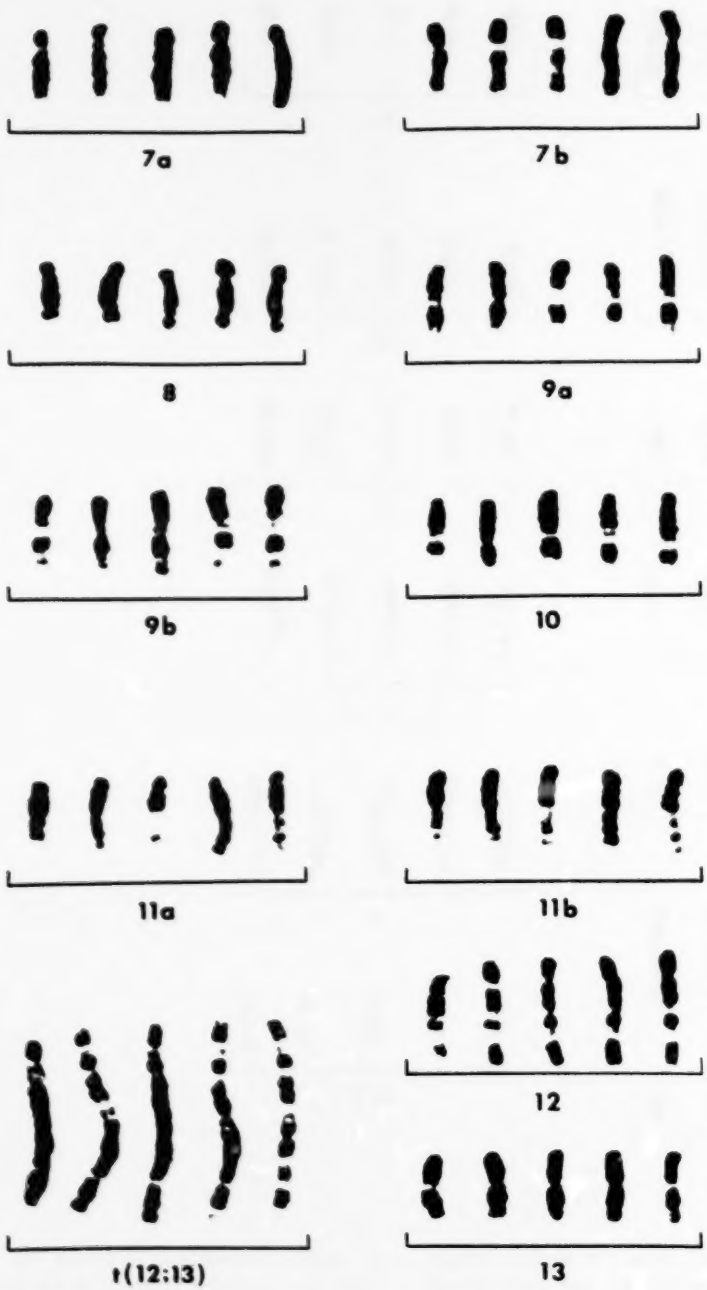

Fig. 3(b).

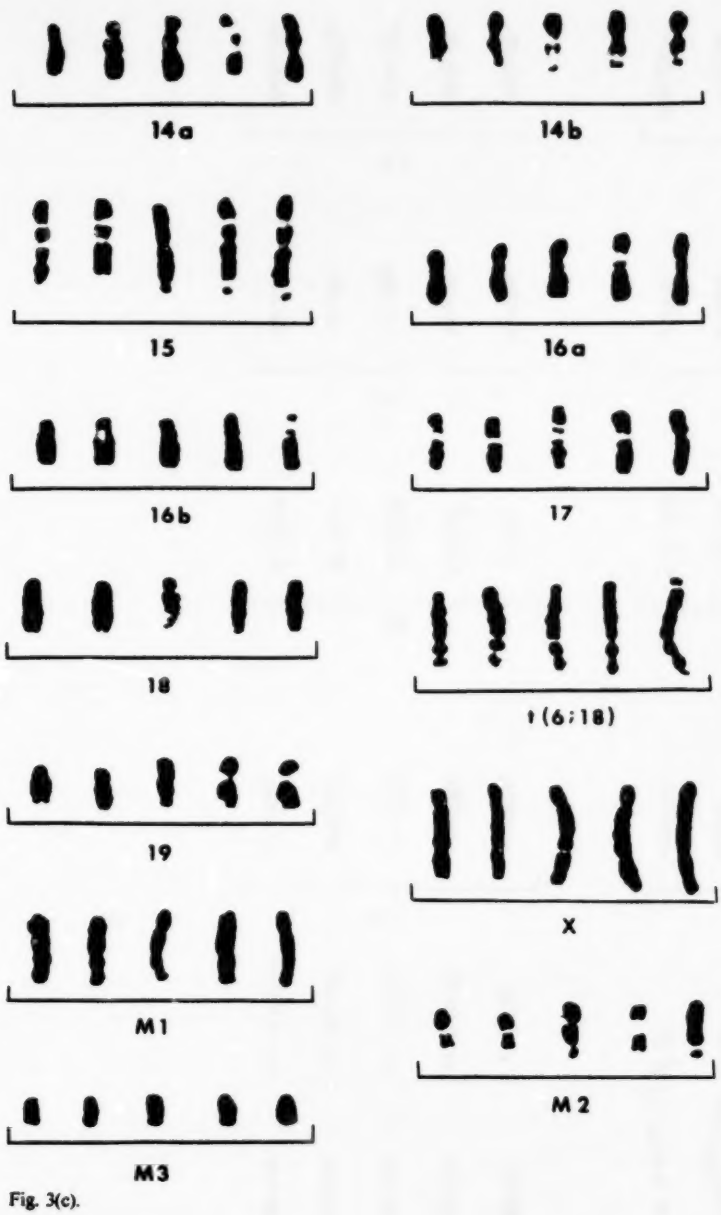

Fig. 3(c).

homologues (11b) which makes it slightly longer (Fig. 3b). This heteromorphism has been noted previously and was used to distinguish between the TK^+ and TK^- chromosomes 11 (Hozier et al., 1982).

Both chromosomes 12 of the $TK^{+/-}$ 3.7.2C cell line have an extra segment at the distal end. This segment appears to contain two G-positive bands and one G-negative band (Fig. 3b). Also, one chromosome 12 is involved in a Robertsonian

translocation (centromere to centromere joining) with one chromosome 13. This fusion produces a very large metacentric chromosome which is the most conspicuous chromosome of the $TK^{+/-}$ 3.7.2C karyotype.

The chromosomes 13 appear to be normal, but as mentioned above, one of them is involved in a centromeric fusion with chromosome 12 (Fig. 3b). The chromosomes 14 have one normal homologue (14a) and one with a centromeric heteromorphism (14b) as well as a terminal deletion at band D1 (Fig. 3c). The chromosome M3 may represent the deleted bands from chromosome 14b.

Only one (abnormal) chromosome 15 is present in the karyotype of the $TK^{+/-}$ 3.7.2C cell line (Fig. 3c). This chromosome is involved in a complex rearrangement with the chromosome 5 and as yet an unidentified chromosomal segment. Bands 5A1–B form the centromeric region of this chromosome, with an intervening chromosomal segment of unknown origin, followed by bands B1–F3 of the chromosome 15.

The chromosomes 16 appear normal except for the absence of part of one of the centromeres in one of the homologues (16b) which makes it slightly smaller than the other homologue (Fig. 3c). The chromosomes 17 both appear normal in the $TK^{+/-}$ 3.7.2C karyotype (Fig. 3c).

Only one normal chromosome 18 is present in the $TK^{+/-}$ 3.7.2C cell line (Fig. 3c). The other chromosome 18 has a translocated segment on the distal long arm. This segment involves bands C3–G of the chromosome 6. Both chromosomes 19 appear normal (Fig. 3c). The $TK^{+/-}$ 3.7.2C cell line has only one normal X chromosome (Fig. 3c).

There are 3 marker chromosomes in the $TK^{+/-}$ 3.7.2C cell line which cannot be assigned as counterparts of the normal karyotype with the present techniques (Fig. 3c).

There appears to be one normal homologue for each pair of $TK^{+/-}$ 3.7.2C chromosomes with the exception of additional material on both chromosomes 12 (Fig. 1), and the complex rearrangement in chromosome 15.

Discussion

With recent advances in chromosome banding techniques, it is now possible to detect subtle changes in the karyotypes of mutagen-treated clonal cell lines, rather than just gross changes in chromosome number. Within the class of chromosome mutations, structural aberrations such as translocations, deletions, and inversions can now be quantified, allowing a more refined evaluation of in vitro genetic bioassay systems. Thus chromosome banding analysis should prove to be a powerful tool for determining the range of mutational events that occur in vitro.

The nomenclature used here for the L5178Y $TK^{+/-}$ 3.7.2C cell line follows the Committee on Standardized Nomenclature of Mice (1972). The use of the schematic representations of chromosomes (ideograms) facilitates the determination of exact break points occurring in structural rearrangements, thus providing a definitive characterization of the chromosomal segments involved. That is, karyotypic changes such as the paracentric inversion present in one chromosome number 7 of the $TK^{+/-}$ 3.7.2C cell line would go undetected without the use of banding techniques. In relation to $TK^{-/-}$ mutant colonies, the ideograms provide a precise method for delineation of additional induced rearrangements.

The analyses presented here and previously (Hozier et al., 1982) indicate the presence of an overall stable karyotype for the $TK^{+/-}$ 3.7.2C heterozygote cell line, containing normal mouse chromosomes, stable chromosome rearrangements, and stable marker chromosomes. The difference between the current chromosomal assignments and those made previously (Hozier et al., 1982) reflect a more precise analysis made possible by more revealing banding patterns. Most significantly, this has resulted in the reassignment of apparent marker chromosomes, previously unrecognizable as rearranged but recognizable mouse chromosomes. There appears to be no karyotypically distinguishable subpopulation among the cells, a situation which would make such analysis considerably more difficult.

The apparent near equality in amount of chromosomal material of DBA/2 mouse chromosomes and that of the $TK^{+/-}$ 3.7.2C cell line indicates that there may have been very little net gain or loss of genetic information during the karyotypic evolution of this cell line. There is at least one normal mouse homologue present for each

chromosome (with the exceptions of a telomeric rearrangement on chromosomes 12 and a paracentromeric rearrangement on chromosome 15). The markers and translocations may prove to be nearly balanced in cytogenetic terms with 'position effects' (Hecht, 1978) rather than net gain or loss of chromosomal material being the more significant change to the genetic constitution of the cell line.

It remains to be determined whether the marker chromosomes are directly derived from the abnormal chromosome homologues in the $TK^{+/-}$ 3.7.2C cell line. It is apparent that the material translocated to the distal end of the chromosome 12 is stable for the $TK^{+/-}$ 3.7.2C cell line and that it has undergone duplication at some point in the evolution of the cell line, since it is the only abnormality appearing in both homologues of any chromosome pair.

To date, analysis of $\sigma TK^{-/-}$ mutants has demonstrated that genetic insult resulting in chromosome 11 aberrations correlates well with the slow-growth kinetics of small-colony mutants (Hozier et al., 1981, 1982). However, the absence of identifiable chromosomal mutations in some small-colony $TK^{+/-}$ mutants (Hozier et al., 1981) indicates the need for more refined analysis. Future work will involve a reexamination of the $TK^{+/-}$ 3.7.2C cell line and its $TK^{-/-}$ mutants at a higher level of chromosome band resolution to determine if some yet undetected damage has occurred in the chromosomes 11 in some small-colony mutants. By analyzing cells at the prometaphase and late prophase stages of chromosome condensation, an increased number of bands can be visualized. High resolution human cytogenetics has already proven useful in finding small deletions, insertions, and translocations which are not detectable at the metaphase level of chromosome analysis (Franke and Oliver, 1978; Yunis et al., 1978). As the examination of $TK^{-/-}$ mutant cell lines continues the use of high resolution cytogenetic techniques may prove useful in elucidating the exact chromosomal nature of some small-colony mutants which by metaphase banding appear normal.

Acknowledgements

The expert technical and editorial assistance of Ms. Joella Pyle, Ms. Diane Goldberg and Ms. Debbie LaFrance is gratefully acknowledged.

References

Clive, D., and J.F.S. Spector (1975) Laboratory procedure for assessing specific locus mutations at the TK locus in cultured L5178Y mouse lymphoma cells, Mutation Res., 31, 17–29.

Clive, D., and Voytek (1977) Evidence for chemically-induced structural gene mutations at the thymidine kinase locus in cultured L5178Y mouse lymphoma cells, Mutation Res., 44, 269–278.

Clive, D., K.O. Johnson, J.F.S. Spector, A.G. Batson and M.M.M. Brown (1979) Validation and characterization of the L5178Y/$TK^{+/-}$ mouse lymphoma mutagen assay system, Mutation Res., 59, 61–108.

Committee on Standardization Genetic Nomenclature for Mice, (1972) Standard karyotype of the mouse, *Mus musculus*, J. Hered., 63, 69–72.

Fisher, G. (1958) Studies of the culture of leukemia cells in vitro, Ann. N.Y. Acad. Sci., 76, 673.

Franke, U., and M. Nesbitt (1971) Identification of the mouse chromosome by quinacrine mustard staining, Cytogenetics, 10, 356–366.

Franke, U., and N. Oliver (1978) Quantitative analysis of high-resolution trypsin-Giemsa bands of human prometaphase chromosomes, Human Genet., 45, 137–165.

Hecht, F. (1978) Are balanced translocations really balanced? Preliminary cytogenetic evidence for position effect in man, Birth Defects Original Article Series, Vol. 14.

Hozier, J.C., and L.L. Lindquist (1980) Banded karyotypes from bone marrow: A clinically useful approach, Human Genet., 53, 205–209.

Hozier, J., J. Sawyer, M. Moore, B. Howard and D. Clive (1981) Cytogenetic analysis of the L5178Y $TK^{+/-} \rightarrow TK^{-/-}$ mouse lymphoma mutagenesis assay system, Mutation Res., 84, 168–181.

Hozier, J., J. Sawyer, D. Clive and M. Moore (1982) Cytogenetic distinction between the TK^{+} and TK^{-} chromosomes in the L5178Y $TK^{+/-}$ 3.7.2C mouse-lymphoma cell line, Mutation Res., 105, 451–456.

Kozak, C.A., and F.H. Ruddle (1977) Assignment of the genes for thymidine kinase and galactokinase to *Mus musculus* chromosome 11 and the preferential segregation of this chromosome in Chinese hamster/mouse somatic cell hybrids, Somat. Cell Genet., 3, 121–133.

Mainguy, P.W., and J.A. Heddle and D.M. Brunette (1975) A reliable method of quantifying G-band positions in chromosomes, Chromosoma, 50, 301–312.

Moore-Brown, M.M., D. Clive, B.E. Howard, A.G. Batson and K.O. Jonnson (1981) The utilization of trifluorothymidine (TFT) to select for thymidine kinase-deficient (TK^{-}) mutants from L5178Y $TK^{+/-}$ mouse lymphoma cells, Mutation Res., 85, 363–378.

Moore, M.M., and D. Clive (1982) The quantitation of $TK^{+/-}$ and $HGPRT^{-}$ mutants of L5178Y/$TK^{+/-}$ mouse lymphoma cells at varying times post-treatment. Environ. Mutagen., 4, 499–519.

Moore, M.M., D. Clive, J.C. Hozier, B.E. Howard, A.G. Batson, N.T. Turner and J. Sawyer (1985) Analysis of trifluorothymidine-resistant (TFT^{r}) mutants of L5178Y/$TK^{+/-}$ mouse lymphoma cells. Mutation Res., 151, 161–174.

Nesbitt, M.N., and U. Franke (1973) A system of nomenclature for band patterns of mouse chromosomes. Chromosoma, 41, 145–158.

Sanchez, O., J.J. Escobar and J.J. Yunis (1973) A simple G-banding technique. Lancet, 1, 269.

Yunis, J.J., J.R. Sawyer and D.W. Ball (1978) The characterization of the high-resolution G-banded chromosomes of man. Chromosoma, 67, 293–307.

NOTICE

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

This article documents research that was conducted under the auspices of the U.S. Environmental Protection Agency (USEPA). As an arm of the government, the USEPA has a paid up, non-exclusive, irrevocable, worldwide license to reproduce, prepare derivative works, and distribute to the public all of the material contained herein, and to grant others acting on its behalf the right to do so. The USEPA grants to the National Technical Information Service, Department of Commerce, the right to act as its agent in the distribution of this material.